REMARKS

The disclosure is objected to because of the following informalities: 1. each page has 2 sets of different page numbers, located at either the bottom center or bottom left of each page; 2. Glucocerebroside is misspelled on page 18 (using the center bottom page number).

Applicants respectfully traverse this rejection because the specification only has page numbers on the bottom center of each page, and Applicants cannot find the misspelling of "glucocerebroside" on page 18 of the specification.

Claims 119-120, 124-125, 127-128, 152-153, 168-170, 182, 196, 199, and 203-204 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner stated the following:

> Claim 119 recites the limitation "glycolipid" in claim 6. There is insufficient antecedent basis for this limitation in the claim.
> Claim 120 recites the limitation "glycolipid" in claim 11. There is insufficient antecedent basis for this limitation in this claim.
> Claims 124-125, 127-128 and 152-153 are rejected for depending from either claim 119 or 120.
> Claim 168 recites the limitation "monosaccharide ceramide" in claim 45. There is insufficient antecedent basis for this limitation in the claim.
> Claim 169 recites the limitation "glucosylceramide" in claim 45. There is insufficient antecedent basis for this limitation in the claim.
> Claim 170 recites the limitation "glucosylceramide" in claim 45. There is insufficient antecedent basis for this limitation in the claim.
> Claim 182 recites the limitation "administering step" in claim 75. There is insufficient antecedent basis for this limitation in the claim.

> Claim 188 recites the limitation "administering step" in claim 109. There is insufficient antecedent basis for this limitation in the claim.
> Claim 196 recites the limitation "mammalian subject" in claim 120. There is insufficient antecedent basis for this limitation in the claim.
> Claim 199 recites the limitation "administering step" in claim 119. There is insufficient antecedent basis for this limitation in the claim.
> Claim 203 recites the limitation "immune-mediated or immune-related disease or disorder" in claim 119. There is insufficient antecedent basis for this limitation in the claim.
> Claim 204 recites the limitation "immune-mediated or immune-related disease or disorder" in claim 120. There is insufficient antecedent basis for this limitation in the claim.

Applicants have amended the claims to address the aforementioned indefiniteness rejections. Therefore, the claims are in proper condition for further examination.

Claims 1, 6, 11, 43-45, 47-52, 54, 59-60, 75-76, 97, 109, 119-120, 124-128, 151-153, 157, 161-166, 168-169, 178-182, 184, 186, 188, 191-196, 199 and 203-204 were rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for, at most treatment of colitis by administration of glucocerebroside, did not reasonable provide enablement for all mammalian diseases. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention commensurate in scope with these claims. Enablement is considered in view of the Wands factors (MPEP 2164.01(a)).

Applicants have made no attempt to cover "all mammalian diseases". Essentially, two groups of disease are believed to be covered by the present invention: cancer and immune modulated diseases. In reference to the latter group, diseases to be treated are included on the basis that they involve an ability to be treated by affecting

the immune response of the patient towards the disease. For example, there are viral infections where the immune response is part of the pathogenic process, autoimmune diseases where again the pathogenic effects are a result of an immune response and metabolic diseases that have defects in immune responses that require remediation by the present invention. This is further exemplified in pending dependent claims of the present application which name a group of diseases that fulfill the above characterizations.

Nature of the Invention. The claims are drawn to a disease treatment of mammalian subject via administration of an intermediary metabolite. Further, the claims are drawn to either inhibition or stimulation in NKT cell number or function caused by either the displacement or the increased binding of activating elements from/to the CD1d molecule.

The claims do not depend upon the function of the CD1d molecule. Potential mechanisms by which the present invention may function were described by Applicants. As such, a way of inhibiting numbers was cited ("This inhibition may be caused by the competitive displacement of activating elements form the CD1d molecule.") Conversely, a way that the NKT cells could be stimulated or increased was also described ("This stimulation may be caused by increased binding of the activating elements form the CD1d molecule."). The invention itself does not depend upon these mechanisms and moreover, these particular mechanisms are only present as dependent claims.

State *of the prior art.* At the time the invention was made, monosaccharide ceramides, such as, glucosylceramides were known in the art to have antitumor and immunostimulatory activities, including suppressing melanoma B16, colon adenocarcinoma and protecting the body against radiation. It was also known in the prior that there are different types of NKT cells, including the CD1d-dependent and the

CD1d-independent NKT cells (see Smyth et al 2002).

The claims of the present invention provide a further limitation that is omitted in the Examiner's analysis. This is that the compounds (including monosaccharide ceramides) must be "intermediary metabolites". Although this language is used throughout the specification there is an additional intrinsic limitation. The present application is a continuation-in part of U.S. Patent Application No. 10/375,906 filed on February 27, 2003 and incorporates it by reference. The disclosure for this application was published on September 2, 2004 as Application No. 2004017152. In this disclosure, particular meaning was given to what constituted an "intermediary intermediate" where it was defined in paragraph [0022] as follows: "In the present invention, metabolites or intermediary metabolites are considered to be the products of enzymatic processes in a mammalian system." As such it is understood that whenever the term "intermediary metabolite" is invoked, it refers to a "mammalian intermediary metabolite". This can also be seen in the Field of the Invention of the specification where it states "The present invention relates to the use of naturally occurring, mammalian intermediary metabolites or T cell receptor ligand...." This is then repeated in the Summary of the Invention of the specification: "The present invention relates to the use of naturally occurring, mammalian intermediary metabolites or T cell receptor ligands...."

The compound cited as an example by the Examiner (Smyth et al.) does not describe an intermediary metabolite as the term is used in the specification. Instead, it describes results achieved with an artificial ligand.

Breadth of the claims. The claims are extremely broadly, encompassing treatment of any and all diseases in any mammal. The claims are not limited to a delivery of a single type of intermediary metabolite, encompassing the use of all intermediary metabolites. Further, an intermediary metabolite can be anything according to the following definition in the specification (paragraph 43) which reads 'the

intermediary metabolite includes, but is not limited to a T cell receptor ligand, a lipid, a polar lipid, a conjugated biomolecule, a glycolipid, a lipoprotein, an apolipoprotein, a glycoprotein, a monosaccharide or polysaccharide ceramide, a glucosylceramide, a galactosylceramide, a glucocerebroside, a glucocerebroside analogue or derivative, a sphingosine, a sphingolipid or a ceramide". The claims do not disclose which population type of NKT cells the invention is drawn to, for example, the CD1d-dependent and the CD1d-independent NKT cells.

Applicants do not agree that the paragraph in the specification states that an intermediary metabolite can be anything, since Applicants have limited it to mammalian intermediary metabolites and have also made an effort to provide a list of specific examples of which intermediary metabolites which could be used in the present invention.

As written, the claims describe the use of NKT cells without describing particular subpopulations. The present invention does not require the stratification of particular subgroups of NKT cells that are involved. The NKT cells are treated as a single group. While the Examiner cites CD1d-dependent and CD-1d independent NKT cells as an example, this would only be necessary if Applicants were trying to further understand mechanisms, which is not an objective of the present invention.

Working examples. Examples in the specification of the instant application reveal the effects of glucocerebroside treatment on only few disease examples, including hepatitis, experimental colitis, melanoma and diabetes on mice. In the colitis example, Applicants evaluated the effects of glucocerebroside by determining the following parameters: diarrhea, degree of colonic ulcerations, intestinal and peritoneal adhesions and wall thickness. There are no working examples directed to the underlying mechanism of glucocerebroside-induced effects of either the CD1d molecule or its activating elements. For example, no binding affinity data is disclosed. While Applicants need not to explain how their invention operates, the absence of such information

makes it impossible to extrapolate the results obtained with glucocerebroside treatment of colitis to the treatment of other diseases with other drugs.

Applicants successfully carried out the extrapolation of results. The examples included in the specification included hepatitis, experimental colitis, melanoma and diabetes. These are diseases that are unrelated in both causation and presentation of symptoms, yet the present invention applies these to a variety of disease due to the presence of a common element: a defect in the immune response of the subjects to the disease. As such, it is expected that other diseases that share this element are likely to also be potential candidates for treatment with the compositions and methods of the present invention. Taniguchi (cited by the Examiner) also contained such extrapolation for use with their compound α-GalCer compound. In addition to the European patent submitted by the Examiner, the same work has been issued as U.S. Patents, including 6,531,453 and 6,747,010. In the latter patent, issued claim 1 begins " A method of treating an autoimmune disease comprising the step of administering...." followed by a generalized description of α-GalCer. As such, their model system was sufficient for a broad claim of "auto-immune disease". Further issued claims were specifically oriented towards systemic lupus erythmatosus and systemic sclerosis although these were not given as Examples.

Knowledge of mechanistic explanations is not necessary in practicing the invention. As seen in the Examples, although this parameter was not determined, beta-glucosylceramide was successfully employed in various model systems.

Guidance in the specification. The specification provides little guidance regarding the practice of the methods as claimed. The specification refers to treatment of specific diseases, many of which were experimentally induced (e.g. colitis), in mice. There is no specific guidance regarding the treatment of all diseases known in all mammalian subjects via administration of all possible intermediary metabolites. Further, the specification provides no evidence regarding the molecular mechanisms of

glucocerebroside-induced effects. The competitive displacement of activating elements from the CD1d molecules and the increased binding of the activating elements to the CD1d molecule are only theories. The specification also fails to provide any evidence showing that the increase or decrease of either number or function of NTK cells is due to the CD1d molecule. The actual activating elements are not disclosed in the specification nor are the type of NTK cells.

The Examiner states that there is no specific guidance for the treatment of all diseases known in all mammalian subjects via administration of all possible intermediary metabolites. With regard to the limited number of examples shown for selected diseases or animal models for diseases, there are usually standard parameters that are commonly used to assess the presence or stage of a disease process that would be readily known by the skilled practitioner. As such, there would not be undue experimentation in applying the compounds that are described in the examples to a disease or an animal model of a disease that is not included in the working examples. Treatment can be carried out and the particular parameters that are associated with a disease or animal model can be carried out in the same way described in the examples. Variations in the particular compounds used by such a practitioner can also be expanded from the specific ones cited in the working examples with reasonable expectations of success.

As the Examiner has previously acknowledged elsewhere, mechanistic explanations are not a requirement. This also applies to the Examiner's comments on causation by the CD1d molecule and the nature of the actual activating elements. Knowledge of the role of these particular elements in the results seen in the examples is not essential for carrying out the present invention.

Predictability of the art. The physiological art in general is acknowledged to be unpredictable (MPEP 2164.03). There is no way to predict the therapeutic effect, if any, of all intermediary metabolites in the treatment of all diseases at the molecular level.

Further, different diseases have different etiologies and different drugs have different modes of action.

Applicants have disclosed an invention that can be used to alter the immune response of an individual to a disease and believe that if it is understood that the invention is being applied to diseases with an immune factor being an essential part of the disease process, it is likely that they may benefit from the present invention. A disease that does not involve an immune aspect would not be a disease of the present invention and would be viewed as unlikely to achieve any benefit from the present invention. Also, it would be known to the skilled practitioner, how much of a factor the immune response contributes towards the disease process. This is a factor that would be understood to directly correlate with the likelihood of therapeutic relief. With regard to the particular intermediary metabolites, the particular ones used in the examples are more likely to be immediately applicable, while a reasoned investigation of similar molecules can be carried out to expand the available repertoire of available reagents. Application of the present invention to other diseases and with other reagents should not require undue experimentation.

Amount of experimentation necessary. Besides the general expectation that it will require years of further research to develop effective therapy for any disease, it would require extensive research to understand the fundamental biology of the each disease. As claimed, essentially all of the work required to ultimately develop a method of treatment has been left for others including determination of a NKT cell type, a specific activating element and the binding affinities of a specific activating element to CD1d molecule.

For the reasons discussed above, it would require undue experimentation for one skilled in the art to use the claimed methods.

The requirement of extensive research to understand the fundamental biology of each disease is not germane to the present invention since it can be said that a

complete understanding of many disease processes is still lacking at the present time but pragmatically speaking, the lack of such complete knowledge has not stopped the development of treatments and procedures for curing or halting such diseases. An understanding of the fundamental biology is only useful in the context where a mechanistic approach to both the disease and the cure is being carried out. Although this is a method that has achieved a degree of success in some circumstances it is not the only way to achieve the development of therapeutic processes.

The list of work required to ultimately develop a method of treatment is research specifically aimed at elucidating the mechanism of action of the present invention. This is not required for the development of treatment modalities.

Claims 1, 11, 43, 54, 59-60, 75, 97, 109, 119-120, 124-128, 151-153, 157, 168-170, 179-182, 184, 186, 188, 199, 203-204 were rejected under 35 U.S.C. 102(b) as being anticipated by Taniguchi et al. (hereinafter referred to as "Taniguchi") The limitations of the above claims are: 1) a method for the treatment of disease in a mammal comprising administration of intermediary metabolite; 2) wherein the result comprises a change, more specifically, an increase in the number or function of regulatory, immune-regulatory or NKT cells; 3) wherein at least one component in the immune system is changed; 4) the method further comprises antigens, including autologous antigens; 5) wherein the intermediary metabolite comprises a monosaccharide ceramide, more specifically, a glucosylceramide; 6) wherein the method of administration is intraperitoneal; 7) wherein the glucosylceramide comprises a glucocerebroside; 8) wherein the glucocerebroside is an analogue or derivative; and 9) wherein the disease or disorder is colitis, more specifically, Ulcerative Colitis.

This prior art reference teaches a treatment method in which glycosylceramides and derivatives are used as the active ingredients in activating NKT cells; this method serves as remedies for diseases and disorders, including ulcerative colitis (whole

document). The structure of glucocerebroside is disclosed on page 3. Further, "antigen presenting cells treated with KRN 7000 showed a marked stimulative effect on Vα24+ NKT cell proliferation in a manner dependent on the number of antigen-presenting cells" (page 18, lines 57-58, also see Figure 9 on page 36). Taniguchi et al disclose the use of autologous antigens in the following quote "an autologous mixed leukocyte reaction (MLR) was performed using these antigen-presenting cells as stimulator cells and autologous peripheral blood mononuclear cells as responder cells" (page 18, paragraph 98). Given that Taniguchi et al meet all of the limitations of the above, these claims are rejected.

Applicants respectfully traverse this rejection. The present application is a continuation-in-part of U.S. Patent Application No. 10/375,906 (hereinafter referred to as the "'906 Application"), which was incorporated by reference. The '906 Application explicitly states "In the present invention, metabolites or intermediary metabolites are considered to be products of enzymatic processes in mammalian systems". Thus, only compounds that have been shown to be products of mammalian enzymatic processes are included in the term "intermediary metabolite". In contrast to the present invention, Taniguchi strictly relates to glycolipids with an alpha linkage between the sugar and the lipid. It should be pointed out that a compound with this linkage is <u>not</u> a mammalian intermediary metabolite as there is no documentation of such compounds ever being isolated from mammalian cells. The compound that is principally used in the examples by Taniguchi is an alpha-linked glycolipid described as KRN7000. On pg 167 of the Smyth et al. reference cited by the Examiner, the following characterization is given: "KRN7000, a marine sponge glycolipid with a novel a-GalCer structure.... ". This compound is specifically characterized as being derived from a sponge. Moreover, the novelty of the structure is in reference to the alpha-linkage, again showing its distinction from glycolipids that are mammalian intermediary metabolites. As pointed out by the Examiner the structure of the Taniguchi compound is shown on page 3. This figure clearly shows the presence of an alpha-linkage instead of the normal mammalian beta

linkage. This point is further emphasized in the text where the compound always includes the prefix "alpha" whenever reference is made to it. Additionally, paragraph [0008] of Taniguchi refers to beta-linked glycosylceramides and in fact teaches away from the use of beta-compounds.

Applicants would like to cite additional references (copies of which are attached herein as Exhibit 3) which provide support that alpha-glycolipids are not found in mammalian cells. Xia et al., (2006) Bioorganic and Medicinal Letters 16; 2195-2199, states on page 2195: "Because α-GalCers were either separated from a marine sponge or chemically synthesized, they are not natural products of mammalian cells". Nakagawa et al. 2001 J Immunol 166; 6578-6584, on page 6583, states: "α-GalCer was originally isolated from a marine sponge (21). No disease or condition has been shown to induce this molecule in mice, suggesting that it is not likely an endogenous ligand in these animals." Hachem et al., 2005 Eur J Immunol. 35; 2793-2802, on page 2794 states: "iNKT cells specifically recognize the exogenous CD1d-bound glycolipid, α-glactosylceramide (α-GalCer), obtained from a marine sponge, but absent from mammalian cells." Spada et al., 1998 J. Exp Med 188; 1529-1534, on page 1529, refers to the Kawano paper describing α-GalCer and states: "The glycolipids that were active in their experiments were composed of an α-anomeric sugar linked to a ceramide type of acylphytosphingosine (APS) lipid, and are structurally related but distinct from the abundant ceramide-containing glycolipids (e.g. gangliosides) found in normal mammalian tissues."

Furthermore, the following two references further support applicants arguments. The abstract in the reference by Parekh et al. 2004 J Immul 173; 3693-3706, states: "Our results show that, contrary to current thinking, β-anomeric GalCer can induce Dc1d-dependent biological activities in mice, albeit lower potency than α-anomeric GalCer." In 2004, those of ordinary skill in the art believed that the NKT effects were unique features of the alpha anomers and not a property of the beta anomers. As such,

effects carried over from studies on the alpha anomer did not render obvious the potential effects by beta anomers. Also, in Kawano et al., 1997 Science 278; 1626-1629, the authors (which were co-inventors of Taniguchi) carried out studies that included certain assays incorporating the use of the beta-anomer of glucosylceramide. Their results showed no effects by the beta anomer, thereby teaching away from the use of the beta anomer. As they concluded on page 1627: "... the α-anomeric conformation of the sugar moiety is essential." They continued this discussion with reference to diglycosylated sugars as well, again concluding that the α-anomeric conformation of the inner sugar (sugar connecting to the ceramide moiety) was an important requirement for activity. The results of this paper showed that the beta anomer showed a lack of activity in the particular assay they used but did not show a lack of utility in other biological assays, as shown in the examples of the present invention.

Claims 1, 6, 11, 43-45, 49-52, 69-60, 97, 119, 124, 161-167, 178-179, 199 and 203 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vliet et al (1999) (hereinafter referred to as "Vliet") and Taniguchi. The limitations of the claims are: 1) a method for the treatment of disease in a mammalian subject comprising administration of an intermediary metabolite; 2) comprising changes in cytokine responses; 3) resulting in a decrease in the number or function of regulatory, immune-regulatory or NKT cells; 4) resulting in a increase in the number or function of regulatory, immune-regulatory or NKT cells; 5) wherein the change in cytokine responses comprise IFN-gamma, IL2, 1L4, ILIO or ILI2; 6) wherein the change in cytokine response comprises a pro-inflammatory, anti-inflammatory or both; 7) wherein the result further comprises changes in the Thl/Th2 balance; 8) intraperitoneally administration of intermediary metabolite and 9) the disease or disorder is colitis.

Vliet et al discloses a method in which NKT B cells isolated from human donors are treated with KRN7000 in culture (see entire document). The alteration in cytokine

profiles are shown in Tables 1 and 2 demonstrating both an upregulation and a downregulation of specific NKT cell functions. More specifically, the Table 1 reveal an upregulation of both pro-inflammatory IFN-gamma and anti-inflammatory IL-4 expression, thus, leading to a change in the Thl/Th2 balance.

Vliet et al does not teach a method for the treatment of colitis in a mammalian subject with intraperitoneally administration of intermediary metabolites. Taniguchi teaches a method in which NKT cell-activating agents, including galatosylceramides or glucosylceramides, are used for therapeutic agents for diseases, including ulcerative colitis (see whole document, including pages 2 and 3). Further, this prior art reference teaches intraperitoneal administration of intermediary metabolites (see page 9, paragraph 39) comprising glucocerebroside and many of its derivatives (see pages 3-6). It would have been obvious to one of ordinary skill in the art to modify the methods taught by Vliet et al and Taniguchi et al in order to alter the cytokine responses via intraperitoneal administration of an intermediary metabolite to treat mammalian disease. One would have motivated to do so, given the suggestion by Vliet et al, because KRN7000 can be recognized by NKT-cells and trigger cytokine release and thus, "be a useful agent in the modulation of immune responses" (see Discussion). There would have been a reasonable expectation of success, given the knowledge that intermediary metabolites are already administered for mammalian treatment, for example, the "α-glucosylceramide structure protects the body from radiation" as well as "increases the number of platelets and leukocytes" (Taniguchi et al, see paragraph 8). Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Applicants respectfully traverse this rejection.

Vliet states the following on page 560:

"Synthetic glycolipids with an α-anomeric structure, including KRN7000, can be recognized by the NKT cell clones and can trigger proliferation, cytokine release (IL-4 and IFN-g) and cytotoxic activity".

This points out that Vliet's work is only concerned with glycolipids with alpha-linkages between the sugar and the lipid. As such, this reference is as deficient as the Taniguchi reference since it also does not involve a mammalian intermediary metabolite. There is no teaching or suggestion in Vliet to indicate that the results obtained with an alpha-linked glycolipid could also be obtained using a beta-linked glycolipid.

Claims 191-196 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vliet et al (1999) and EP 0 988 860 (2000, hereinafter as "Taniguchi"), and further in view of Connolly and Cunningham (2000). As mentioned above, Vliet et al. and Taniguchi combined teach a method for the treatment of colitis in a mammalian subject with intraperitoneally administration of intermediary metabolites. These references do not disclose food and/or water deprivation prior to administration of intermediary metabolites. This practice is, however, commonly taught in the prior art by many references including that by Connolly and Cunningham. It would have obvious to one of ordinary skill in the art to modify the methods taught by Vliet et al, Taniguchi and Connolly and Cunningham to incorporate fasting prior to the administration of intermediary metabolites. One would have been motivated to do so, given the suggestion by Connolly and Cunningham, in order to minimize the volume and increase the pH of the gastric contents. There would have been a reasonable expectation of success, given this practice has been advised since the late 19th century (see Connolly and Cunningham). Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Applicants respectfully traverse this rejection.

Firstly, since Applicants have shown that Vliet and Taniguchi combined do no teach a method for the treatment of colitis in a mammalian subject with intraperitoneal administration of intermediary metabolites, it would not have been obvious to one of ordinary skill in the art to modify the methods taught by Vliet, Taniguchi and Connolly and Cunningham to incorporate fasting prior to the administration of intermediary metabolites. Secondly, one would not have been motivated to do so in order to minimize the volume and increase the pH of the gastric contents, because that is not the reason Applicants require fasting prior to administration of the intermediary metabolite. Applicants require fasting because many foods contain certain amounts of various intermediary metabolites that may cause potential unwanted positive and negative effects which would interfere with the desired effect of the treatment.

SUMMARY

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejections of record and further examination of the amended claims. These claim amendments have not resulted in the addition of new matter. Early and favorable action is respectfully requested.

No other fee or fees are believed due in connection with this paper. In the event that any fee or fees are due, however, the United States Patent and Trademark Office is hereby authorized to charge any such fee or fees to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney requests that she be contacted at the number provided below.

Respectfully submitted,

Natalie Bogdanos
Registration No. 51,480
Attorney for Applicants

ENZO LIFE SCIENCES, INC.
c/o ENZO BIOCHEM, INC.
527 Madison Avenue, 9th Floor
New York, New York 10022-4304
Telephone: (212) 583-0100
Facsimile: (212) 583-0150

al/USProsecution/Enz-64(CIP).amendment.05.01.07